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Genetic and morphological variation in the parthenogenetic earthworm *Eiseniella tetraedra* (Sav.) (Oligochaeta: Lumbricidae) from South Finland and North Norway

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Summary. Sixteen overall enzyme phenotypes (clones) were identified in 179 *Eiseniella tetraedra* individuals from southern Finland and from northern Norway about 1200 km to the north. The common clones were unevenly distributed between the localities. The two most frequent clones A and B were recorded both in the north and in the south. Using these as norm clones, comparisons showed that the clones in N Norway deviated on average less from clone A than from clone B. The opposite was true for the clones in S Finland. Diversity of clones in the pooled Norwegian sample was higher than in the corresponding Finnish sample. Similarity of clones was greater within northern and within southern than between northern and southern localities. *E. tetraedra* is tetraploid (4n = 72) in the study area. Polyploidy and the pattern of clonal variation indicate that it is also parthenogenetic. Size and segment numbers in adults varied according to locality but no clear-cut regional trend was found. Male pore terminalia were present in all the adults and they usually located on the 13th segment. In northern worms they were more protuberant than in southern ones. Morphological variability showed no correlation to the enzyme patterns. Even though *E. tetraedra* is stenotopic, in addition to being parthenogenetic and polyploid, it shows rather wide morphological and genetic diversity between populations which may be attributed to the impacts of mutations, dissimilar dispersal events and environmental variables.

Key words: Earthworm, parthenogenesis, *Eiseniella*, electrophoresis, enzyme, clone

Introduction

Many Lumbricidae species reproduce by facultative or obligate parthenogenesis (e.g. Christensen 1980; Casellato 1987). Triploid and tetraploid strains of the obligatorily parthenogenetic *Eiseniella tetraedra* have been recorded in southern Europe and England (Muldal 1949, 1952; Omodeo 1952, 1955; Vedovini 1973). In obligatorily parthenogenetic earthworms there is no recombination of alleles, the main source of novel gene combinations in amphimictic species. In Europe (e.g. Plisko 1973) and in USA (Gates 1977) *E. tetraedra* shows great morphological variability. In eastern Fennoscandia it is a stenotopic species mostly occupying waterlogged habitats such as shores (Terhivuo 1988a). We have earlier surveyed allozymic and morphological variability in the eurytopic and obligatorily

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parthenogenetic earthworm *Dendrobaena octaedra* (Sav.) in eastern Fennoscandia (Terhivuo 1988b; Terhivuo & Saura 1990). Now we aim to assess genotype affinities and morphological variation in the stenotopic *E. tetraedra* collected in the same region.

Materials and methods

Localities and sample material

We sampled three *E. tetraedra* populations in northern Norway and two in southern Finland about 1200 km south of the former area (Fig. 1). Loc. 5 is situated in the hemiboreal and loc. 4 in the southern boreal vegetation zone whereas the Norwegian sample sites (1 – 3) refer to the northern boreal zone (Ahti et al. 1968). The localities and habitats are:

1 *Norway, Ifjordfellet:* The bank of a subarctic mountain brook with low (20 cm) *Salix*-bushes and patches of grasses and herbs. The mull-like soil made up a 1 – 2 cm thick layer on the parent material comprising gravel and clay. The field layer coverage by Graminae, *Ranunculus*, *Alchemilla* and *Rumex* spp. was c. 80% and mosses were abundant (ground layer coverage c. 80%), too. The pH of the soil was 5.3 (destilled water/soil = 1 : 1).

2 *Norway, Berlevåg:* The bank of a freshwater brook situated some hundred meters from the seashore. The worms were under *Salix* bushes among organic material brought ashore by spring floods. The organic soil was dense grass turf with parent material of gravel, sand and some clay mixed with them. Grasses and herbs (field layer coverage c. 80%, *Deschampsia*, *Alchemilla* spp.) and mosses (ground layer coverage c. 80%) were abundant on the bank. The soil pH was 6.0.

3 *Norway, Vardö:* A bank of a freshwater brook close to the seashore. The worms were extracted from under mosses (coverage c. 20%) with mull-like 1 – 2 cm thick soil layer and sand underneath. The coverage of grasses and herbs (total c. 90%, *Carex*, *Juncus*, *Achemilla* spp.) was dense but mosses covered only c. 20% of the field layer. The pH of the soil was 6.5.

4 *Finland, Inkoo, Bruksträsket:* The worms were found among mixed deciduous and coniferous leaf litter brought ashore by waves of the lake during fall flood. The litter was water-logged and it was lying on sand. In the upper part of the shore there was mull soil and dense *Alnus* thicket. The pH of the litter was 5.6.

5 *Finland, Tuulos, Oksjärvi:* The worms were sampled close to the lake shoreline in soil with 1 – 2 cm thick mull and heavy clay underneath. There were *Alnus* bushes (coverage c. 70%) and field layer with dense field layer vegetation (coverage c. 90%, *Rubus*, *Urtica*, *Filipendula* and *Graminae* spp.) but less dense coverage of mosses (c. 20%). The pH of the soil was 5.4.

The Norwegian worms (n = 99) were sampled in August 1990 and the Finnish worms (n = 80) in September 1990. Localities 2 and 3 were situated some meters above the sea level whereas loc. 1 is c. 500 m, loc. 4 c. 90 m and loc. 5 c. 10 m above the sea level. Most of the 179 worms were adults. For the numbers of worms in each locality see the legend to Fig. 1. During the fieldwork the worms from a locality were kept together in a plastic jar provided with an air supply and cooled in cold water containers. In the laboratory they were kept in jars at +10 °C for some days. In the jars they laid several cocoons which were collected and used for chromosome analysis and inspection of morphological traits in newly hatched juveniles. The vials with cocoons were inspected every 1 – 2 day and hatched juveniles were immediately fixed in 70% ethanol. Prior to weighing the adults were washed with tap water and dried on paper towels for about one minute. Depending upon the size of the worms a set of 5 – 19 anterior segments was cut off and stored in −70 °C until headed to electrophoresis some months later. The rest of the worm was fixed in 70% ethanol for morphological inspection.

Enzyme systems and morphological traits

The following enzymes were polymorphic in our assays; Pgi (phosphoglucose isomerase), Pgm (phosphoglucomutase), Mdh (malate dehydrogenase), Idh (isocitrate dehydrogenase), G-3-pdh (glyceraldehyde-3-phosphate dehydrogenase) and Lap (leucine aminopeptidase). The overall enzyme phenotype (briefly clone) for each individual worm was determined over all these loci. Even though a clone may be a heterogeneous grouping, we assume that the worms with the same overall enzyme

phenotype are genetically more closely related to each other than to the worms of the other clones. We also assayed some 20 other enzyme systems but they proved to be either monomorphic or poorly reproducible and we therefore excluded them from the final definition of the clones. The starch gel electrophoresis and enzyme assays were used as decribed by Saura et al. (1979). We examined the following somatic and reproductive characters in adults; fresh body weight (live specimens with gut

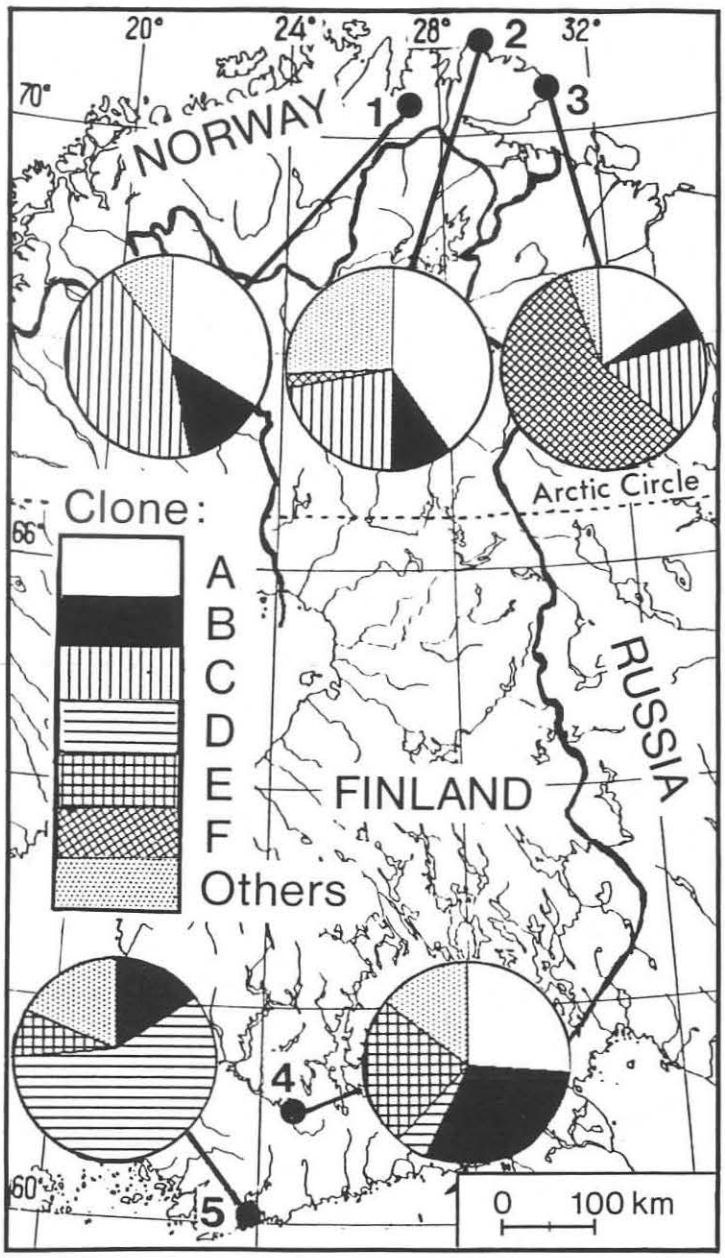

Fig. 1. Sample localities and proportions of the most common *E. tetraedra* clones. 1. Ifjordfellet (n = 30), 2. Berlevåg (n = 50), 3. Vardö (n = 19), 4. Tuulos, Oksjärvi (n = 42) and 5. Inkoo, Brukträsket (n = 38). For the clones see the text

contents included), total number of segments (taking into account those used for electrophoresis), posterior length (= the length for clitellum + posterior part of the body measured to the nearest 0.5 mm in individuals preserved in ethanol), location and protuberance of clitellum, tubercula pubertatis and male pore terminalia. In juveniles we counted segment numbers and measured body length to the nearest 0.5 mm in individuals fixed in ethanol.

Chromosome analysis

The chromosomes were studied in mitotic metaphases from embryos (or unhatched juveniles) taken from the cocoons. Prior to preparation the cocoons were kept at +4 °C for at least one day. The embryos were fixed in acetic acid methanol (1 : 3) for 30 minutes and softened in 60% acetic acid. The cell suspension was spread on a microscopic slide on a hot plate (+50 °C). The preparations were stained with 5% Giemsa solution.

Statistics

We estimated clone pool diversity in a random sample of 50 *E. tetraedra* individuals from S Finland and N Norway by means of the rarefaction index. This distribution-free method avoids the difficulty of dissimilar sample size by calculating the number of clones for random samples smaller than the original sample catch (Simberloff 1978, 1979, see also Terhivuo & Saura 1990). In measuring clone pool similarities we applied the formula of Ochiai (1957) (see also Terhivuo & Saura 1990). This method, however, gives equal weight to every clone in common for the two sites compared irrespective of the number of individuals belonging to a clone. In order to consider this viewpoint we also calculated clone pool similarities according to the Renkonen Number based on relative abundances of the clones in each site. For the index the lower relative abundance value of each clone present in both of the sites were summed up and arranged in a hierarchial relationship (for the details see Wallwork 1970, pp. 212–221). Because the frequency distributions of the morphological traits surveyed deviate from the corresponding normal distributions we applied non-parametric statistical tests when comparing them. Symbols for statistical significances are: *** = $P \leqq 0.001$, ** = $0.01 \geqq P > 0.001$, * = $0.05 \geqq P > 0.01$, o = $0.10 \geqq P > 0.05$ and ns = $P > 0.10$.

Results

Genetic variability

Allozyme variation: The number of enzyme variants in the polymorphic enzyme systems ranged from 2 to 4 (Table 1). Most differences between the clones were due to variability observed in Pgi, Pgm and Lap whereas in Mdh, Idh and G-3-pdh variants were represented by few individuals. It is noteworthy that some common variants such as Pgi/2 and Pgm/6 occurred only in the Norwegian worms whereas Lap/18 recorded in the Finnish worms was absent in them (Tables 1 and 2). We identified 16 clones from the total sample catch of 179 individuals. Accordingly, about every 11th individual belonged on the average to a new clone. About 94% of all the worms belonged to frequent clones, i.e. to those with at least 5 individuals. Clones recorded only in one locality comprised 44% of all the clones (Tables 1 and 2) while the five clones represented by a single individual made up about 31% of all the clones and about 3% of all the individuals. The numbers of individuals representing the common clones imply that *E. tetraedra* is parthenogenetic in the area studied (Table 2). In an amphimictic species each genotype is usually represented by only one individual.

Table 1. Enzyme variants and compilations of variants for *E. tetraedra* clones (= overall enzyme phenotype)

Enzyme system	Serial no for variant	Occurrence of variants			Enzyme variant compilation (Pgi-Pgm-Mdh Idh-63pdh-Lap)	Symbol for clone	Number of indiv.	
		indiv.		popul.				
		%	n	n			n	%
Pgi	1	85.5	(153)	5	1-7-9-11-13-16	A	44	24.6
	2	9.5	(17)	2	1-5-9-11-13-16	B	29	16.2
	3	1.1	(2)	1	1-6-9-11-13-16	C	27	15.1
	4	3.9	(7)	2	1-5-9-11-13-18	D	24	13.4
Pgm	5	40.8	(73)	5	1-5-9-11-13-17	E	13	7.3
	6	16.2	(29)	3	2-7-9-11-13-16	F	12	6.7
	7	40.8	(73)	5	1-7-9-11-13-17	G	9	5.0
	8	2.2	(4)	1	4-7-9-11-13-16	H	6	3.4
Mdh	9	99.4	(178)	5	1-5-9-11-13-19	I	5	2.8
	10	0.6	(1)	1	2-8-9-11-14-16	J	3	1.7
Idh	11	99.4	(178)	5	3-6-9-11-13-16	K	2	1.1
	12	0.6	(1)	1	1-5-9-11-14-16	L	1	0.6
6-3-pdh	13	96.1	(172)	5	1-5-10-11-15-17	M	1	0.6
	14	3.3	(6)	2	2-7-9-11-13-17	N	1	0.6
	15	0.6	(1)	1	2-8-9-12-14-16	O	1	0.6
Lap	16	70.4	(126)	5	4-7-9-11-14-16	P	1	0.6
	17	13.4	(24)	4				
	18	13.4	(24)	2	Total	16	179	
	19	2.8	(5)	2				

Distributions of clones: Table 2 shows that there are non-randomly distributed clones. For instance, clones D and E were recorded only in the two localities of S Finland whereas clones C and F were absent there but present in three and two of the Norwegian localities, respectively. Supposing random distribution of individuals between the localities the probabilities for some of the distributions indicated in Table 2 for individuals of a clone by chance are significantly low $\left(\text{clone D: } \left(\frac{80}{179}\right)^{24}, \text{E: } \left(\frac{80}{179}\right)^{13}, \text{C: } \left(\frac{99}{179}\right)^{27}, \text{i.e. } P < 0.001\right.$ for all three clones and for F: $\left.\left(\frac{99}{179}\right)^{12}, P < 0.008\right)$. Table 2 also shows that the two most common clones (A and B) are widely distributed within the regions. In S Finland there are 11 and in N Norway 33 individuals of clone A. The corresponding numbers for clone B are 19 and 10. In spite of being present in most localities the frequencies of these clones are dissimilar in the two countries ($\chi^2 = 11.85^{***}$, df = 1).

Clone pool adaptation: To survey whether the clone associations in N Norway and in S Finland show any regional adaptation we adopted the two most common clones A and B as norm clones. Our hypothesis is that if *E. tetraedra* clone pools are regionally adapted, the enzyme variant compilations of the clones in N Norway should approach differently the norm clones than the enzyme variant compilations of the clones in S Finland. We accordingly grouped the clones with respect to the number of enzyme variants in which they differed from clone A. Another classification was based on the difference to clone B. Because the number of individuals deviating in 3 or 4 enzyme variants from the corresponding norm was low (< 7) we pooled them with those differing in 2 enzyme variants from the corresponding norm. In N Norway about 86% of the worms differed in one enzyme variant from clone A and about 14% in 2 or more variants. In S Finland the

Table 2. *E. tetraedra* clones recorded in the five sample localities. 1. Ifjordfellet, 2. Berlevåg, 3. Vardö, 4. Tuulos, Oksjärvi, 5. Inkoo, Fagervik, Brukstäsket, 1.–3. N Norway and 4.–5. S Finland. For the symbols of the clones see Table 1

	Norway (locs. 1–3)						S Finland (locs. 4–5)					
	1		2		3		1–3		4		5		4–5	
Clone	%	n	%	n	%	n	%	n	%	n	%	n	n	%
A	33.3	(10)	40.0	(20)	15.8	(3)	33.3	(33)	26.2	(11)	–	–	13.8	(11)
B	13.3	(4)	10.0	(5)	5.3	(1)	10.1	(10)	31.0	(13)	15.8	(6)	23.8	(19)
C	43.3	(13)	22.0	(11)	15.8	(3)	27.3	(27)	–	–	–	–	–	–
D	–	–	–	–	–	–	–	–	4.8	(2)	57.9	(22)	30.0	(24)
E	–	–	–	–	–	–	–	–	23.8	(10)	7.9	(3)	16.3	(13)
F	–	–	2.0	(1)	57.9	(11)	15.0	(12)	–	–	–	–	–	–
G	–	–	4.0	(2)	–	–	2.0	(2)	11.9	(5)	5.3	(2)	8.8	(7)
H	10.0	(3)	6.0	(3)	–	–	6.1	(6)	–	–	–	–	–	–
I	–	–	2.0	(1)	–	–	1.0	(1)	–	–	10.5	(4)	5.0	(4)
J	–	–	6.0	(3)	–	–	3.0	(3)	–	–	–	–	–	–
K	–	–	4.0	(2)	–	–	2.0	(2)	–	–	–	–	–	–
L	–	–	–	–	–	–	–	–	2.4	(1)	–	–	1.3	(1)
M	–	–	–	–	–	–	–	–	–	–	2.6	(1)	1.3	(1)
N	–	–	–	–	5.3	(1)	1.0	(1)	–	–	–	–	–	–
O	–	–	2.0	(1)	–	–	1.0	(1)	–	–	–	–	–	–
P	–	–	2.0	(1)	–	–	1.0	(1)	–	–	–	–	–	–
Total:														
n of ind.	30		50		19		99		42		38		80	
n of clones	4		11		5		12		6		6		8	

corresponding percentages differed significantly from these (Table 3, $\chi^2 = 33.76^{***}$, df = 1). When clone B is used as the norm, individuals deviating in 1 enzyme variant are relatively more frequent in S Finland than in Norway while those differing in 2–4 variants are less frequent there than in N Norway (grouping II, Table 3). The difference between the two regions is statistically significant ($\chi^2 = 6.68^{**}$, df = 1). We conclude that in the boreal zone *E. tetraedra* clone pools do not comprise a random array of clones.

Table 3. Percentages and number of *E. tetraedra* individuals deviating in 1 or 2–4 enzyme variants from the two most frequent clones A and B in N Norway and S Finland. For additional information see the text

No of deviating allozymes	Grouping I (deviation from clone A)				Grouping II (deviation from clone B)			
	Norway		Finland		Norway		Finland	
	%	(n)	%	(n)	%	(n)	%	(n)
1	86.4	(57)	62.3	(26)	68.5	(61)	86.9	(53)
2–4	13.6	(9)	37.7	(43)	31.5	(28)	13.1	(8)
Total	100.0	(66)	100.0	(69)	100.0	(89)	100.0	(61)

Table 4. Coincidence table for *E. tetraedra* clone pools in pairs of sites and countries as shown by the index of Ochiai (left lower half) and Renkonen (right upper half or the matrix). For the symbols of the localities see Fig. 1 and for the indices see statistics

Loc.	1	2	3	1–3	4	5
1	–	0.71	0.37	–	0.40	0.13
2	0.60	–	0.39	–	0.40	0.12
3	0.67	0.54	–	–	0.21	0.05
1–3	–	–	–	–	–	0.34
4	0.41	0.37	0.37	0.35	–	0.34
5	0.20	0.37	0.18	0.35	0.67	–
4–5	0.35	0.43	0.32	0.41	–	–

Diversity of clone pools: The number of clones varies according to the locality and the country (Table 2). In loc. 2 clonal diversity is higher than elsewhere. The two Finnish samples are rather similar. Pooling the samples according to country and applying the rarefaction method, a higher number of clones can be expected for a random sample of e.g. 50 individuals in N Norway ($\bar{x} \pm SD = 9.4 \pm 1.2$) than in S Finland ($\bar{x} \pm SD = 7.2 \pm 0.7$).

Similarity of clone pools: Clone pool diversities do not indicate anything about the clones shared by the populations. We focused on this viewpoint by comparing the sites as shown in Table 4. The similarity indices calculated between the Norwegian localities ranged from 0.54 to 0.67 (Ochiai index) and from 0.37 to 0.71 (Renkonen index) and between the Finnish localities it was 0.67 and 0.34, respectively. Corresponding comparisons between the Norwegian and the Finnish localities as well as between the samples pooled according to country showed a much lower level of similarity (Ochiai index: 0.18 and 0.41, respectively). Accordingly, it seems that the three northern populations are on average more closely related to each other than to the two investigated in the south and *vice versa*.

Chromosome studies: The somatic chromosome number was 72 counted from embryos from four cocoons laid by worms from S Finland (loc. 5). The same chromosome number was also found in four other embryos produced by individuals from N Norway (loc. 1). The chromosome complement consists of 18 groups of four homologous chromosomes each (Fig. 2). The worms are, accordingly, tetraploid with the basic chromosome number $n = 18$ in our study area.

Morphological variability

Body size: We used fresh body weight and posterior length (clitellum + posterior part of the body) as indices for body size. In adults these traits are correlated ($r_s = 0.73^{***}$, $n = 157$). In the two countries the adults are homogeneous as for body weight and posterior length, but the localities in turn proved to be significantly heterogeneous in these respects (Tables 5 and 6). To point the localities responsible for the dissimilarity we applied *a posteriori* statistics to the mean ranks received from the Kruskal-Wallis tests (for the procedure see Zar 1984, p. 200–201). Fresh body weight of the adults of locs. 2–4 were similar to each other and also in locs. 1 and 5. Concerning posterior length locs. 1, 4 and 2 were similar and also locs. 5 and 3 were similar. We conclude that there are local differences in adult body size but no clearcut regional trend exists. In order to study whether the differences in body size of adults are already present in their juvenile offspring we measured

Fig. 2. The tetraploid chromosome complement of *Eiseniella tetraedra*. The basic chromosome number is n = 18. The 72 chromosomes are arranged in 18 groups of four homologous chromosomes each. The chromosomes are monocentric: four groups of metacentric, eight groups of submetacentric and six groups of acrocentric chromosomes

body lengths of newly hatched juveniles (Table 7). As these juveniles were not older than 1–3 days and they were not given any food we assume that their body lengths are mainly due to prenatal growth.

As in the case of adults, S Finland and N Norway were similar but differences between localities existed. *A posteriori* tests indicated that actually none of the localities differed significantly from the others. This implies that factors related to prenatal development do not play a major role in the adult body size in *E. tetraedra*.

Segment numbers: An earthworm grows by increasing the size and number of segments. Posteriorly, segments may fuse or new segments may form (Edwards & Lofty 1972). We counted segment numbers in newly-hatched juveniles as well as in the adults that produced

Table 5. Fresh weight of *E. tetraedra* adults. For the localities (1–5) see the legend to Fig. 1. Symbols: $\bar{X} \pm SD$, = mean ± standard deviation

Loc.	Fresh body weight (g)			
	$\bar{X} \pm SD$	Median	Range	n
1	0.121 ± 0.030	0.111	0.078–0.181	23
2	0.132 ± 0.034	0.129	0.066–0.249	46
3	0.122 ± 0.027	0.126	0.058–0.158	13
1–3	0.127 ± 0.032	0.126	0.058–0.249	82
4	0.163 ± 0.051	0.126	0.090–0.158	41
5	0.100 ± 0.022	0.102	0.049–0.143	34
4–5	0.134 ± 0.051	0.120	0.049–0.296	74
Kruskal-Wallis:				
between localities			***, df = 4	
between countries			ns, df = 1	

Table 6. Posterior length (mm, clitellum + posterior part of the body) of ethanol fixed *E. tetraedra* adults. For the symbols see Fig. 1 and Table 5

Loc.	Posterior length			
	$\bar{X} \pm SD$	Median	Range	n
1	22.7 ± 2.8	22.0	18.0–28.5	23
2	21.0 ± 3.4	20.8	14.0–33.0	46
3	19.0 ± 2.3	19.0	15.5–22.0	13
1–3	21.1 ± 3.3	21.0	14.0–33.0	82
4	22.7 ± 4.5	22.0	16.5–35.0	41
5	18.3 ± 2.4	18.3	13.0–23.5	34
4–5	20.7 ± 4.3	19.5	13.0–35.0	75
Kruskal-Wallis:				
between localities			***, df = 4	
between countries			ns, df = 1	

Table 7. Body length (mm) of newly hatched *E. tetraedra* individuals (ethanol fixed material). For the symbols see Fig. 1 and Table 5

Loc.	Body length			
	$\bar{X} \pm SD$	Median	Range	n
1	7.6 ± 0.4	7.5	6.5 ± 8.5	22
2	7.3 ± 0.6	7.0	6.0 ± 8.5	22
3	6.8 ± 0.9	7.0	5.5 ± 8.0	9
1–3	7.3 ± 0.6	7.5	5.5 ± 8.5	53
4	7.2 ± 0.6	7.5	6.0 ± 8.5	19
5	6.5	6.5	6.5	2
4–5	7.1 ± 0.6	7.0	6.0 ± 8.0	21
Kruskal-Wallis:				
between localities			**, df = 4	
between countries			ns, df = 1	

them. Table 8 shows the heterogeneity of the study material in this respect. The juveniles had dissimilar numbers of segments in different localities and countries implying that prenatal processes are involved. The adults from locs 1 and 5 differed from each other and all the other adults whereas in locs. 2–4 the adults had similar segment numbers as shown by *a posteriori* tests applied. With the exception of loc 1 pairwaise comparisons between newly hatched juveniles and adults from the same locality show that the juveniles had higher mean and median numbers of segments. Amputation of segments postnatally can contribute to the dissimilarity between adults and juveniles. It still remains obscure why the difference in the skewness of the distributions and the medians between juveniles and adults vary so much according to locality. For instance the difference between the medians ranged from 2 to 10 (Table 8). Because we have excluded all the adults with observable amputations such great differences hardly can be attributed to occasional amputations. We rather suggest that postnatal fusion of segments is also involved as observed in *L. terrestris* by Evans (1946).

Table 8. Segment numbers in newly hatched *E. tetraedra* juveniles and adults. For the localities see Fig. 1. Symbols: cf. Tables 4 and 6. Md = median, skewness of distributions are according to Pearson's index: 3 × (mean-median)/standard error

Loc.	Juveniles				Adults				Juv/Ad (Kruskal-Wallis)	
	$\bar{X} \pm SD$	Md	Skew-ness	n	$\bar{X} \pm SD$	Md	Skew-ness	n	P	df
1	83.8 ± 16.9	87.0	−0.6	22	85.3 ± 4.5	85.0	+0.2	23	ns	1
2	87.2 ± 3.5	88.0	−0.7	22	77.2 ± 10.0	80.5	−1.0	46	***	1
3	80.0 ± 5.6	83.0	−1.2	10	76.5 ± 7.6	77.0	+0.4	13	*	1
1–3	86.1 ± 4.5	86.0	+0.1	54	79.4 ± 9.2	82.0	−0.9	82	***	1
4	85.0 ± 3.3	86.0	−0.9	19	75.9 ± 8.3	80.0	−1.5	41	***	1
5	83.0 ± 1.4	83.0	0.0	2	70.7 ± 9.9	74.0	−1.0	34	***	1
4–5	84.8 ± 3.2	86.0	−1.1	21	73.5 ± 9.3	76.0	−0.8	75	***	1
Kruskal-Wallis:			Juv.				Ad.			
between localities			***, df = 4				***, df = 4			
between countries			***, df = 4				***, df = 1			

Table 9. Estimated protuberance and location of male pore terminalia in the five sample localities. Scale: P1 = male pore terminalia not very prominent, P2 = rather prominent and P3 = very prominent and well-demarcated

Loc.	Protuberance of male terminalia				Male terminalia on segment			
	P1	P2	P3		XIII		XV	
	%	%	%	(n)	%	(n)	%	(n)
Norway:								
1	71	29	–	(24)	100	(24)	–	–
2	16	67	16	(49)	95	(41)	5	(8)
3	–	31	69	(13)	100	(13)	–	–
Finland:								
4	93	7	–	(42)	100	(42)	–	–
5	94	6	–	(34)	100	(34)	–	–

Male pore terminalia and clitellum: None of the *E. tetraedra* adults totally lacked male pore terminalia. In 95% they were located on the 13th and in 5% on the 15th segment. All adults with male pore terminalia on the 15th segment originated from loc. 2 where they made up 16% of the adults. Another adult worm from loc. 1 had male pore terminalia on the left side of the 12th segment but on the right side it was on the 13th segment. One adult (loc. 2) lacked a male pore on the right side but had it on the left side of the 13th segment. A third adult (loc. 1) had a male pore on the left side on 13th segment but on the right side it had two pores, viz. on the 13th and the 15th segment. The protuberance of male pores varied according to locality to some extent but in the Finnish worms they were on average less prominent than in the Norwegian worms (Table 9). In most adults the clitellum was located on segments 22–27 but also worms having it on segments 23–27 and 22–26 were found. Other variants were represented by only one individual each. We also noticed some within segment variability in this respect but the data are not shown. The frequency of the adults with clitellum on segments 23–27 and that of the other variants is similar in the two countries ($\chi^2 = 0.601$, df = 1) (Table 10). Tubercula pubertatis are located on segments 23–26 in 87% of the adults but also those having them on segments

Table 10. Location of clitellum and tubercula pubertatis in *E. tetraedra* adults from the five sample localities

On segments	loc 1		loc 2		loc 3		Norway		loc 4		loc 5		Finland	
	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)
Clitellum:														
22–27	92	(22)	90	(44)	62	(8)	86	(74)	95	(40)	85	(29)	92	(79)
23–27	4	(1)	8	(4)	31	(4)	10	(9)	–	–	3	(1)	1	(1)
22–26	–	–	–	–	8	(1)	1	(1)	2	(1)	12	(4)	6	(5)
others	4	(1)	2	(1)	–	–	2	(2)	2	(1)	–	–	1	(1)
Tubercula pubertatis:														
23–26	88	(21)	84	(41)	85	(11)	85	(73)	90	(38)	82	(28)	87	(66)
23–25	8	(2)	16	(8)	–	–	12	(10)	7	(3)	18	(6)	12	(9)
others	4	(1)	–	–	15	(2)	3	(3)	2	(1)	–	–	1	(1)

23–25 were rather common (12%). The frequencies of the worms with tubercula pubertatis on segments 23–26 and those having it on other segments is the same in the two countries ($X^2 = 0.619$, df = 1). In one adult from loc. 1, in three from loc. 4 and in one from loc. 5 empty spermatophores were present on segments 18–21. In three of them the spermatophores were attached on both sides of the body, but in two worms they were present only on the left side. They were situated near or on setal lines a or b or in the intersegmental furrow of these setal lines. Accordingly, adults with spermatophores occurred in the north and in the south but the function of spermatophores, if any, remains unknown.

Morphological traits in relation to clones and clone groups

Body size and segment numbers show great heterogeneity between the common clones (Table 11). We furthered the analysis by grouping the adults of clones A, B and C according to the country and loçality (Table 12). Unfortunately, these are the only clones with at least two localities where the number of the adults is >5. The Kruskal-Wallis test applied indicates that regional and local differences vary according to the clone and the character involved and no clearcut trend can be found. We also tried to find out regional differences between the adults of the clone groups (Table 12). Both in groupings I and II the clone categories proved to be homogeneous with regard to body size but heterogeneous as to segment numbers. The mean and median segment numbers proved to be higher in N Norway than in S Finland. The eight adults with male pore terminalia on the 15th segment belonged to clones A (3 indiv.), B (1), C (2), O (1) and P (1) showing that this variety is not confined to any single genotype. The adults with spermatophores in turn represented clones C (1), E (2) and G (2).

Discussion

Genetic divergence of clones

Our definition of *E. tetraedra* clones is based on six polymorphic enzyme systems. These enzymes are much the same we used in surveying *Dendrobaena octaedra* (Terhivuo & Saura 1990). In these two species the numbers of the variants in the same enzyme system is, however, different. Parthenogenesis in combination with polyploidy should allow an

Table 11. Mean (± standard deviation) and median (Md) fresh weight (g), posterior length (mm) and segment numbers in adults of the common *E. tetraedra* clones (A-H)

Clone	Weight			Posterior length			No of segments		
	$\bar{X} \pm SD$	Md	n	$\bar{X} \pm SD$	Md	n	$\bar{X} \pm SD$	Md	n
A	0.140 ± 0.045	0.130	42	21.87 ± 3.72	21.5	42	77.8 ± 9.3	80.0	42
B	0.132 ± 0.050	0.129	28	20.79 ± 4.39	20.5	28	77.3 ± 9.9	81.0	28
C	0.127 ± 0.036	0.119	20	21.27 ± 3.98	21.0	20	81.3 ± 9.1	84.5	20
D	0.106 ± 0.031	0.102	24	18.31 ± 2.26	18.0	24	68.6 ± 9.0	70.5	24
E	0.152 ± 0.044	0.149	13	22.50 ± 3.27	22.0	13	78.6 ± 6.4	80.0	13
F	0.122 ± 0.023	0.125	7	18.71 ± 2.43	19.0	7	74.1 ± 8.5	75.0	7
G	0.137 ± 0.049	0.123	9	21.44 ± 5.49	23.0	9	74.2 ± 10.0	76.0	9
H	0.133 ± 0.031	0.142	5	22.20 ± 3.11	21.0	5	79.8 ± 12.3	84.0	5
Kruskal-Wallis:									
P	**			***			***		
df	7			7			7		

Table 12. Mean and median fresh weight (g), posterior length (mm) and segment numbers in clones A and B and in the two clone groupings. For further explanation see the text

Clone/clone group and country/loc	Fresh weight		Posterior length		Segment number		
	$\bar{X} \pm SD$	Md	$\bar{X} \pm SD$	Md	$\bar{X} \pm SD$	Md	n
Clone A							
Norway:	0.130 ± 0.033	0.122	21.3 ± 3.0	21.5	78.8 ± 9.1	82.0	31
– loc 1	0.117 ± 0.034	0.106	22.6 ± 3.2	22.0	83.2 ± 5.4	84.0	10
– loc 2	0.138 ± 0.033	0.139	21.0 ± 2.9	21.0	76.1 ± 10.5	77.5	18
– loc 3	0.120 ± 0.012	0.116	19.0 ± 1.0	19.0	80.3 ± 2.9	82.0	3
Finland: (= loc 4)	0.170 ± 0.063	0.147	23.4 ± 5.1	21.5	74.9 ± 9.7	79.0	11
Kruskal-Wallis							
between localities	ns, df = 4		ns, df = 4		ns, df = 4		
between countries	ns, df = 1		ns, df = 1		ns, df = 1		
Clone B							
Norway:	0.130 ± 0.036	0.139	21.6 ± 3.5	21.0	82.4 ± 7.6	85.0	10
– loc 1	0.123 ± 0.029	0.120	22.0 ± 2.9	21.5	85.0 ± 2.4	85.0	4
– loc 2	0.151 ± 0.020	0.144	22.4 ± 3.2	21.0	79.4 ± 10.1	82.0	5
– loc 3	0.058	–	15.5	–	87.0	–	1
Finland:	0.133 ± 0.057	0.124	20.4 ± 4.9	19.3	74.4 ± 10.1	80.0	18
– loc 4	0.155 ± 0.055	0.144	21.5 ± 5.2	19.5	75.2 ± 10.1	80.0	12
– loc 5	0.088 ± 0.030	0.081	18.1 ± 3.5	18.8	73.0 ± 10.8	75.5	6
Kruskal-Wallis							
between localities	*, df = 4		ns, df = 4		***, df = 4		
between countries	ns, df = 1		ns, df = 1		**, df = 1		
Clone C							
Norway:	0.127 ± 0.036	0.119	21.3 ± 4.0	21.0	81.3 ± 9.1	84.5	20
– loc 1	0.128 ± 0.027	0.126	23.1 ± 2.5	22.0	87.7 ± 3.4	87.0	7
– loc 2	0.123 ± 0.043	0.113	20.1 ± 4.7	19.5	77.4 ± 10.3	81.0	11
– loc 3	0.144 ± 0.021	0.144	21.8 ± 0.4	21.8	80.5 ± 2.1	80.5	2
Kruskal-Wallis							
between localities	ns, df = 3		*, df = 3		*, df = 3		
Grouping I:							
– Norway (1)	0.126 ± 0.033	0.126	20.9 ± 3.7	21.0	79.8 ± 9.6	83.0	44
– Finland (1)	0.137 ± 0.054	0.125	20.9 ± 5.0	19.5	74.5 ± 9.4	77.0	25
– Norway (2–4)	0.126 ± 0.021	0.126	21.7 ± 1.7	21.5	78.7 ± 6.9	80.0	7
– Finland (2–4)	0.126 ± 0.042	0.111	19.8 ± 3.3	19.0	72.5 ± 9.4	76.0	39
Kruskal-Wallis							
between countries	ns, df = 3		ns, df = 3		***, df = 3		
Grouping II:							
– Norway (1)	0.128 ± 0.034	0.121	21.3 ± 3.4	21.0	79.8 ± 9.1	82.0	51
– Finland (1)	0.133 ± 0.050	0.116	20.6 ± 4.0	19.5	73.0 ± 9.4	76.0	50
– Norway (2–4)	0.123 ± 0.026	0.126	20.5 ± 3.0	21.0	76.9 ± 9.7	79.0	21
– Finland (2–4)	0.149 ± 0.047	0.150	22.4 ± 5.5	23.5	74.7 ± 7.9	76.0	7
Kruskal-Wallis							
between countries	ns, df = 3		ns, df = 3		**, df = 3		

extensive accumulation of mutations (Suomalainen et al. 1987). In our study the majority of *E. tetraedra* clones deviated only in 1 or 2 enzyme variants from the two most common clones A and B. Such differences probably have arisen through mutations in an evolving lineage, even though the direction of evolution in these enzyme systems is not clear. Table 1 implies that the more a clone differs from the norm clones A or B the lower is the number of individuals belonging to it and the smaller is the geographical range it occupies. Perhaps mutations produce clones with a lower capacity for dispersal and survival compared to that of the norms. Alternatively, the clones that are inferior in numbers of individuals to those of the norms may have recently arrived and not yet completely established themselves there.

The clones studied here are, of course, clone groups as only a fraction of the total genotype was exposed to electrophoresis. Enzyme differences are here used merely as markers for clones. Such markers can be useful in defining ecologically dissimilar clones or even species in parthenogenetic Oligochaeta (Christensen 1980; Øien & Stenersen 1984) but they need not correlate with morphological traits. Depending on the polymorphic enzyme system adopted, partly or completely overlapping ranges or separate distributions for individual clones can be recorded. This point is discussed in detail in Terhivuo & Saura (1990). In this context we recall the fact that enzyme systems like Pgi, Pgm and Lap with at least two common variants showed clear-cut geographical differences for the worms having different variants in their genotype. These enzyme systems are accordingly taken as efficient markers for clones.

Clone pool adaptation

The diversity of a clone pool in a region can be taken as a measure for the genetical potential of a parthenogentic population to cope with its environment. The more diverse the clone pool is the better the population can adapt and maintain itself in a habitat. Based on six polymorphic enzyme systems Terhivuo & Saura (1990) showed that in a sample of 428 *D. octaedra* individuals every third worm represented a new genotype. In *E. tetraedra* about every 11th individual represented a novel genotype. Because the enzyme systems underlying the definition of the clones on the two species were not equal, we defined the clones once more on the basis of Pgi, Pgm, Mdh, Idh and Lap enzymes which were polymorphic in both species. Application of rarefaction method showed that in a random sample of 100 *E. tetraedra* individuals only 12.3 ± 0.98 ($\bar{X} \pm$ SD) clones can be expected. The corresponding expectation in *D. octaedra* was 28.3 ± 2.9 ($\bar{X} \pm$ SD). We conclude that *D. octaedra* shows considerably higher clonal diversity than *E. tetraedra* in the study area.

A survey on eurytopic *D. octaedra* (Terhivuo & Saura 1990) showed that clone pool diversity increased northwards in open habitats but decreased in forests. In open biotopes some of the pattern may be explained by postglacial dispersal of *D. octaedra* clones from the west. Table 2 shows that in *E. tetraedra* clone pool diversity as a whole is higher in N Norway than in S Finland. This is, however, due to the sample from loc. 2 (Table 2). The two other Norwegian localities do not differ much from those in S Finland in this respect. Clone similarities between nearby localities can be due to migration of clones to and from neighbouring areas. This could explain why *E. tetraedra* populations in the north share more northern clones and in the south more southern clones in comparison to what the two major areas share with each other. *E. tetraedra* is an opportunistic species with good capacity for dispersal. This is evidenced by its wide distribution in uninhabited subarctic regions where human impact is negligible. Ability to disperse over wide areas can promote similarities between clone pools whereas effective selection can lead to diversification. In this survey clone pool similarities in *E. tetraedra* are on an average higher than in *D. octaedra* in which no similarity value (Ochiai index) between the populations exceeded 0.5 (Terhivuo & Saura 1990).

Chromosome number and parthenogenesis

As only embryonic material was cytogenetically investigated we have no data about the meiosis in *E. tetraedra*. The tetraploid somatic chromosome number 72 agrees with the observations of Muldal (1949, 1952) from the British Isles and with those of Omodeo (1955) from Italy and Turkey. Triploid chromosome numbers have been reported for this species in other localities in Italy (Omodeo 1952) and in Provence in southern France (Vedovini 1973). The basic chromosome complement (n = 18) with eight submetacentric, four metacentric and six acrocentric chromosomes (Fig. 2) resembles that in the hexaploid species *Dendrobaena octaedra* (Hongell & Terhivuo 1989). On the basis of cytological evidence (Černosvitov 1930) and individuals reared in isolation (Janda & Gavrilov 1939) *E. tetraedra* is parthenogenetic. In this survey the clone pattern of variation indicates that this species is parthenogenetic also in our study area. The spermatophores present in some *E. tetraedra* adult worms were empty flaps varying in size even in the same worm. Spermatophores have been recorded in this species in USA (Gates 1977), England (Sims & Gerard 1985) and Turkey (Omodeo & Rota 1989). They probably have no reproductive function. It may be a remnant phase of behaviour from an earlier amphimictic way of reproduction.

Variability and evolution of morphological traits

Parthenogenetic earthworm species descend from amphimictic forms and they exhibit wide morphological variability (e.g. Stephenson 1930). Terhivuo (1988a) demonstrated in *Dendrobaena octaedra* that adults in N Norway and N Finland are larger than in S Finland no matter which biotope or overall enzyme phenotype was considered. The number of segments was, however, similar in the whole study area. In addition, postnatal growth takes place in *D. octaedra* mainly by increasing the size of segments and no remarkable increase or decrease in postnatal segment numbers was observed. As for reproductive organs about 40% of *D. octaedra* adults and subadults lacked visible male pore terminalia and southern adults possessed more prominent clitellum and tubercula pubertatis than their northern allies. The number of segments was very heterogeneous among *E. tetraedra* adults and juveniles in different populations. Pooling the present material and comparing it with data from Canada (Gates 1977) a wide overlap in segment numbers is observed even though the populations are significantly dissimilar (Canada: $\bar{X} \pm SD = 81.2 \pm 5.6$, median = 83, range = 60–89, n – 56, Finland and Norway: $\bar{X} \pm SD = 76.6 \pm 9.7$, median = 80, range = 52–93, n = 157, P = 0.002, Kruskal-Wallis P = 0.002, df = 1). In *Allolobophora caliginosa trapezoides* (Duges) dissimilarities in segment numbers between populations are mainly due to extrinsic factors (Omodeo & Magaldi 1951) and depend on the amount of food available for the embryo or the juvenile when still within the cocoon. This does not, of course, exlude the role of genetic control of the reaction shown.

Gates (1977) showed that in most *E. tetraedra* adults obtained from different parts of the world the clitellum is on segments 22–27. Less than 10% of the worms had a clitellum on segments 23–27, 23–26 or 22–26. The adults with tubercula pubertatis on segments 23–26 were most frequent in his material but variants having it on 23–25, 24–25 and 24–26 were recorded, too. According to Table 10, a clitellum on segments 22–27 and tubercula pubertatis on segments 23–26 is also the mode for *E. tetraedra* in our material. Male pore terminalia are situated on the 13th segment (f. *typica*) in most *E. tetraedra* individuals originating from different parts of Europe (Plisko 1973; Zajonc 1980) and from Canada (Gates 1977). Adults having them on segments 15 (f. *hercynia*) are also rather often present, whereas individuals having them on segments 12 or 14 are rare. Pool (1937) lists the latter two forms as genovarieties to explain their sporadic occurrence. Our material is concordant with this view; mutations can cause "abnormalities" both in the location and the numbers of male pore terminalia in individual worms representing dissimilar genotypes and coming from separate localities. One may also recall that one *E. tetraedra*

possessed two male pores on segments 13 and 15 on the right side of the body. We could not correlate clonal differences to morphological variability in *E. tetraedra*. For comparable cases in other groups of parthenogenetic animals see Suomalainen et al. (1987). In parthenogenetic organisms each clone is selected as a whole. Morphological traits related to the male reproductive system which does not seem to have any function in *E. tetraedra* need not possess any particular adaptive value for the species. Such traits may vary without being subject to any selection. The clonal variability observed may, however, have adaptive value to the species. The wide geographical range and clonal diversity shows that the two species have clone pools that allow them to maintain populations even in far-away regions with very dissimilar environmental variables. It is also noteworthy that even though *E. tetraedra* is a stenotopic species its dispersal capacity does not seem to be inferior to that of the widely eurytopic *D. octaedra*.

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References

Ahti, T., Hämet-Ahti, L., Jalas, J. (1968) Vegetation zones and their sections in northwestern Europe. Ann. Bot. Fennici **5**, 169–211

Casellato, S. (1987) On polyploidy in Oligochaetes with particular reference to lumbricids. In: Bonvicini Pagliai A. M., Omodeo, P. (eds), On earthworms. Selected. Symposia and Monographs U. Z. I., 2. Mucchi Editore, Modena

Černosvitov, L. (1930) Studien über die Spermaresorption. I. Teil. Die Samenresorption bei den Oligochaeten. Zool. Jahrb. Abt. Anat. Ontog. Tiere **52**, 487–538

Christensen, B. (1980) Annelida. Animal cytogenetics **2**, 1–81

Edwards, C. A., Lofty, J. R. (1972) Biology of earthworms. Chapman and Hall, London

Evans, A. C. (1946) Distribution of numbers of segments in earthworms and its significance. Nature, London **158**, 98–99

Gates, G. E. (1977) Contribution to a revision of the earthworm family Lumbricidae. XX. The genus *Eiseniella* in North America. Megadrilogica **3** (5), 71–80

Hongell, K., Terhivuo, J. (1989) Chromosomal status of the parthenogenetic earthworm *Dendrobaena octaedra* (Sav.) (Oligochaeta: Lumbricidae) in southern Finland. Hereditas **10**, 179–182

Janda, V., Gavrilov, K. (1939) Untersuchung über die Vermehrungsfähigkeit von Individuen einiger Oligochaeten-Arten, die schon vor Erreichung der Geschlechtsreife isoliert wurden. Vest. Zool. Pol. Praze **6–7**, 254–256

Muldal, S. (1949) Cytotaxonomy of British earthworms. Proc. Linnean Soc. **161**, 116–118

Muldal, S. (1952) The chromosomes of earthworms I. The evolution of polyploidy. Heredity **6**, 55–76

Ochiai, A. (1957) Zoogeographical studies on the solenoid fish found in Japan and its neighbouring regions. II. Bull. Japan Soc. Sci. Fisheries **22**, 26–530

Omodeo, P. (1952) Caryologia dei Lumbricidae. Caryologia **4**, 173–275

Omodeo, P. (1955) Cariologia dei Lumbricidae. II. Contributo. Caryologia **8**, 135–178

Omodeo, P., Magaldi, A. (1951) Variabilita nei gemelli univolari di lomrico. Sci. Genetica **4**, 13–21

Omodeo, P., Rota, E. (1989) Earthworms of Turkey. Boll. Zool. **56**, 167–199

Plisko, J. D. (1973) Lumbricidae. Dzdzownice (Annelida: Oligochaeta). Fauna Polski **1**, 1–156

Pool, G. (1937) *Eiseniella tetraedra* (Sav.). Ein Beitrag zur vergleichenden Anatomie und Systematik der Lumbriciden. Acta Zool., Stockholm **18**, 1–110

Saura, A., Lokki, J., Oura, E., Suomalainen, H. (1979) Qualitative yeast enzyme analysis by electrophoresis. Eur. Journ. Appl. Microbiol. Biotechnol **7**, 355–364

Simberloff, D. S. (1978) Use of rarefaction and related methods in ecology. In: Dickson, K. L., Garins, J. Jr., Livingston, J. (eds) Biological data in water pollution assessment: quantiative ans statistical analysis. Am. Soc. Testing and Materials, STP 652

Simberloff, D. S. (1979) Rarefraction as a distribution free method of expressing and estimating diversity. In: Grassle, J. F., Patil, G. P., Smith, W. K., Taille, C. (eds) Ecological diversity in theory and practice. Fairland, Maryland

Sims, R. W., Gerard, B. M. (1985) Earthworms. Synopsis British Fauna (New series) **31**, 1–171
Stephenson, J. (1930) The Oligochaeta. Clarendon Press, Oxford, 978 pp
Suomalainen, E., Saura, A., Lokki, J. (1987) Cytology and evolution in parthenogenesis. CRC Press, Boca Raton, Florida
Terhivuo, J. (1988a) The Finnish Lumbricidae (Oligochaeta) fauna and its formation. Ann. Zool. Fennici **25**, 229–247
Terhivuo, J. (1988b) Morphological and morphometric variation of the parthenogenetic earthworm *Dendrobaena octaedra* (Sav.) (Oligochaeta, Lumbricidae) in eastern Fennoscandia. Ann. Zool. Fennici **25**, 303–320
Terhivuo, J. & Saura, A. (1990) Allozyme variation in parthenogenetic *Dendrobaena octaedra* (Sav.) Oligochaeta: Lumbricidae) populations of eastern Fennoscandia. Pedobiologia **34**, 113–139
Vedovini, A. (1973) Systematique, caryologie et ecologie des oligochaetes terrestres de la region Provencale, These Univ. Provence
Wallwork, J. A. (1970) Ecology of soil animals. McGraw-Hill, London
Zajonc, I. (1980) New knowledge on the genus *Eiseniella* (Lumbricidae). Biologia (Bratislava) **35**, 341–348
Zar, J. H. (1984) Biostatistical analysis. Prentice-Hall, New Jersey
Øien, N., Stenersen, J. (1984) Esterases of earthworms III. Electrophoresis reveals that *Eisenia fetida* (Savigny) is two species. Comp. Biochem. Physiol. **78 C**, 277–278